Applicant	Focus Diagnostics, Inc. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
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Summary Date	July 24, 2012
Proprietary Name	**STRATIFY JCV® DxSelect™**
Generic Name	JCV ELISA
Classification	Class II, Special Controls § 21 CFR 866.3336, Anti-JCV antibody detection assay. Product Code: OYP
Predicate Devices	STRATIFY JCV™ Antibody ELISA

AUG 16 2012

INTENDED USE

The Focus Diagnostics' STRATIFY JCV® DxSelect™ assay is intended for the qualitative detection of antibodies to JC virus in human serum or plasma. The assay is intended for use in conjunction with other clinical data, in multiple sclerosis patients receiving or considering natalizumab therapy, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only.

The assay is not intended for donor screening. The performance of this assay has not been established for use in other immunocompromised patient populations or patients with different disease conditions or undergoing other treatments or in neonates and pediatric patient populations.

SUMMARY AND EXPLANATION OF THE TEST

Progressive multifocal leukoencephalopathy (PML) is an opportunistic infection of the central nervous system (CNS) caused by JC virus (JCV), a polyoma virus, that is pathogenic only in humans. It is thought that exposure to the JC virus occurs early in life (pre-adolescence) and recently published studies have reported that approximately 50% to 60% of adults have been infected with JCV, as evidenced by the presence of antibody to JCV in the serum. In rare instances, the virus reactivates and progresses to PML such as in individuals who are immune compromised. Treatment with immunomodulatory therapies increase the risk of developing PML in JCV infected individuals. While JCV exposure is necessary for the development of PML, the development of the disease is also dependent on both host and viral factors, as well as immune status. Since JCV infection is a necessary step for PML development, an assay to detect JCV exposure in patients may be a potentially useful tool to stratify patients for PML risk (i.e., identifying patients who may be at a lower or higher risk of developing PML).

There is an increased risk of PML in natalizumab treated multiple sclerosis (MS) and Crohn's disease (CD) patients. Three risk factors for PML have been identified in MS and CD patient populations: natalizumab treatment duration, prior immunosuppressant use, and the presence of antibodies to JCV. Utilizing these three risk factors, sub-groups of patients can be identified with both higher and lower risk for PML. Please consult the current, locally available, prescribing or supplemental physician information for natalizumab (Tysabri®) for detailed information on the known risks associated with JCV serological status and the development of PML in natalizumab-treated patients. The patient's JCV antibody status should be considered in combination with other known PML risk factors when evaluating the benefit and risk of initiating or continuing therapy with natalizumab. Patients with all three known risk factors have the highest risk for the development of PML.

TEST PRINCIPLE

In the Focus Diagnostics' STRATIFY JCV® DxSelect™ test, JC virus-like particles (VLP) are pre-coated onto 96-well microtiter plates. Diluted serum or plasma specimens and controls are incubated in the wells to allow JCV-specific antibodies present in the specimens to react with the JC VLP antigen. Nonspecific reactants are removed by washing. Peroxidase-conjugated anti-human antibodies are added to react with JCV-specific antibodies. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD). Specimen OD readings are compared with Cut-Off Calibrator OD readings to determine results. Each specimen result is reported as an index value. A specimen with an index value that is greater than a specified upper cut-off is reported as positive for detectable JCV-specific antibodies, whereas a specimen with an index value less than the specified lower cut-off is reported as negative for detectable JCV-specific antibodies. A specimen with an index value that is equal to or between the upper and lower cut-off values is reported as indeterminate. An indeterminate result requires further evaluation in the confirmation (inhibition) assay.

In the confirmation assay, soluble JC VLP antigen will compete with plate bound JC VLP antigen for the JCV-specific antibodies present in the serum or plasma specimens. After washing away the unbound antibodies, peroxidase-conjugated anti-human antibodies are added and bind to any captured JCV-specific antibodies. Excess conjugate is removed by washing. Enzyme substrate and chromagen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of OD. The percent inhibition is calculated to confirm presence of JCV-specific antibodies in the specimen. Specimens with a percent inhibition value that is greater than the specified cut-off are reported as positive for detectable JCV-specific antibodies, whereas specimens with percent inhibition values less than or equal to the cut-off are reported as negative for detectable JCV-specific antibodies.

Comparison to Predicate

Item	Device	Predicate
Name	STRATIFY JCV® DxSelect™	STRATIFY JCV™ Antibody ELISA
Intended Use	The Focus Diagnostics' STRATIFY JCV® DxSelect™ assay is intended for the qualitative detection of antibodies to JC virus in human serum or plasma. The assay is intended for use in conjunction with other clinical data, in multiple sclerosis patients receiving or considering natalizumab therapy, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only. The assay is not intended for donor screening. The performance of this assay has not been established for use in other immunocompromised patient populations or patients with different disease conditions or undergoing other treatments or in neonates and pediatric patient populations.	The STRATIFY JCV™ Antibody ELISA testing service provided by Focus Diagnostics is intended for the qualitative detection of antibodies to John Cunningham Virus in human serum or plasma. The assay is intended for use in conjunction with other clinical data, in multiple sclerosis and Crohn's disease patients receiving natalizumab therapy, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only and is to be performed only at Focus Diagnostics' Reference Laboratory. The assay is not intended for donor screening. The performance of this assay has not been established for use in other immunocompromised patient populations or in neonates and pediatrics patient populations.
Assay Methodology	Detection ELISA with a secondary Confirmation ELISA for specimens that have results between the two cut-off levels.	Detection ELISA with a secondary Confirmation ELISA for specimens that have results between the two cut-off levels.
Assay Target	Antibodies to John Cunningham Virus (JCV)	Antibodies to John Cunningham Virus (JCV)
Sample Type(s)	Serum and plasma	Serum and plasma

Item	Device	Predicate
Name	STRATIFY JCV® DxSelect™	STRATIFY JCV™ Antibody ELISA
Sample Preparation – Detection Assay	1:101 dilution in sample diluent	1:200 dilution in sample diluent
Sample Preparation – Confirmation Assay	1:101 dilution in confirmation diluent	1:200 dilution in assay diluent spiked with JCV antigen.
Assay Cut-Offs	Index < 0.2, negative	nOD < 0.1, negative
	Index ≥ 0.4, positive	nOD ≥ 0.2, positive
Assay Preparation	ELISA plates are pre-coated, all buffers are pre-mixed. Calibrators and controls require dilution before use.	User must coat ELISA plates prior to use and mix specific reagents. Controls require dilution before use.
Antigen	JC Virus Like Particles	JC Virus Like Particles
Antibody	Peroxidase-conjugated donkey anti-human JCV bodies	Peroxidase-conjugated donkey anti-human JCV bodies

Clinical Performance (Comparative Agreement)

Because PML is an infrequent event in natalizumab-treated patients, data collected from both clinical trial and post-marketing reports of confirmed cases of PML were used to assess the clinical performance of the STRATIFY JCV® DxSelect™ assay for PML risk stratification. A clinical plan was developed for collection of serum samples obtained from natalizumab-treated patients prior to the onset of PML for JCV antibody testing. A total of 31 available serum samples from confirmed PML patients collected at least 6 months prior to clinical diagnosis of PML were tested for JCV antibody status using the STRATIFY JCV® DxSelect™ assay. In addition to the pre-PML samples, 1330 Samples from MS patients were tested by the STRATIFY JCV® DxSelect™ assay. A total of 707 patients receiving treatment tested positive for antibodies to JCV using the test, and the positivity rate was estimated to be 58.7% (414/707) with a 95% CI of 54.9% to 62.1%. An assay is statistically informative if the percentage of positive results in patients with the disease of interest is higher than the percentage of positive results in the population at risk. The 100% JCV antibody positivity demonstrated in the 31 natalizumab-treated PML patients prior to PML diagnosis was significantly different than the 58.7% JCV antibody positivity in the MS population, and represents an approximately 2-fold increased risk of PML compared to the PML incidence in the overall natalizumab-treated population.

In prior clinical studies for natalizumab the risk of developing PML was estimated using statistical modeling. The relative risk for patients who have received natalizumab for at least 18 months is shown in the table below. Risk was calculated based on statistical modeling with an assumption that there is one hypothetical PML case with a negative test result (38 PML cases: 37 positive and 1 hypothetical negative) and an assumption that the study has 13,227 patients.

Table 1: Estimated incidence of PML by JCV serological status

		Number with PML	Number without PML	Total number patients treated
JCV Serological Status	Positive	37	7,229	7,266
	Negative	1*	5,960	5,961
Total		38	13,189	13,227
Risk of PML (per 1,000) treated with ≥ 18 months for Positive result		5.09 95% CI: 3.70 to 7.01		
Risk of PML (per 1,000) treated with ≥ 18 months for Negative result		0.17 95% CI: 0.03 to 0.95		
Relative risk		30.4 95% CI: 5.3 to 437.4		

*For the negative result, a hypothetical case was assumed in order to allow for calculation of the point estimate.

The studies demonstrated that the positivity rate for JCV antibodies is not dependant upon prior immunosuppressant (IS) use or the duration of natalizumab treatment. The results of the STRATIFY JCV® DxSelect™ assay can be used along with other established PML risk factors, of prior IS use and natalizumab treatment duration, to stratify an individuals risk for PML, please refer to Table 1 and to the prescribing information for additional risk estimates.

Performance with pre-PML samples.

The STRATIFY JCV® DxSelect™ assay was compared to a cleared Anti-JCV assay (STRATIFY JCV™ Antibody ELISA) using serum samples obtained from PML patients at least 6 months prior to PML diagnosis. Because PML is an infrequent event in natalizumab-treated patients, data collected from both clinical trial and post-marketing reports of confirmed cases of PML were used to assess the clinical performance of the STRATIFY JCV® DxSelect™ assay for PML risk stratification. Thirty-one available serum samples from confirmed PML patients collected at least 6 months prior to clinical diagnosis of PML were tested at one internal testing site for JCV antibody status using the STRATIFY JCV® DxSelect™ assay and the validated laboratory methodology. The sample set demonstrated 100% (31/31) positive agreement (95% CI: 89.0% to 100%) with the validated assay.

Performance with archived clinical specimens

Two groups of prospectively collected and archived clinical samples obtained from the STRATIFY-2 and the AFFIRM clinical studies were used to assess the performance of the STRATIFY JCV® DxSelect™ assay compared to the validated laboratory methodology used in the STRATIFY-2 and AFFIRM clinical studies. One group consisted of patients who were receiving natalizumab, the other group of patients had not received natalizumab therapy (and were considering receiving natalizumab). The samples were blinded and randomly distributed to two external testing sites and one internal testing site. The data was analyzed for each group separately. Results for the two individual groups are presented in the tables below. Positive Percent Agreement was greater than 97% for each group and Negative Percent agreement was greater than 90% for each group.

Table 2: Agreement for Clinical Samples – Patients Receiving Natalizumab

STRATIFY JCV® DxSelect™	Cleared Anti-JCV Assay		Total
	Positive	Negative	
Positive	385	29	414
Negative	12	281	293
Total	397	310	707
Positive Percent Agreement (PPA)	97.0% (385/397) 95% CI: 94.8 to 98.3%		
Negative Percent Agreement (NPA)	90.6% (281/310) 95% CI: 86.9 to 93.4%		

Table 3: Agreement for Clinical Samples - Patients Considering Natalizumab

STRATIFY JCV® DxSelect™	Cleared Anti-JCV Assay		Total
	Positive	Negative	
Positive	326	24	350
Negative	5	268	273
Total	331	292	623
Positive %Agreement (PPA)	98.5% (326/331) 95% CI: 96.5 to 99.4%		
Negative %Agreement (NPA)	91.8% (268/292) 95% CI: 88.1 to 94.4%		

Positivity Rate and Expected Values:

The STRATIFY JCV® DxSelect™ assay been used to evaluate JCV antibody positivity rate in serum and plasma samples from a geographically diverse cohort of 1330 MS patients. The cohort was comprised from MS patients from clinical trials including a completed Phase 3 clinical study of natalizumab in MS patients (AFFIRM C-1801), an ongoing study to evaluate seroprevalence in the MS population (STRATIFY-2 [101JC402). The clinical characteristics for the MS patients within each study are shown in Table 4. The age and gender distribution of the MS cohort tested with the STRATIFY JCV® DxSelect™ are similar to the age and gender distribution of MS patients treated with natalizumab in the post-marketing setting. JCV antibody positivity rate in the MS cohort was 55-59% which is consistent with what has been reported in the literature. JCV antibody positivity rate was shown to increase with age and was lower in women compared to men which is also consistent with what has been reported in the literature in healthy adults using similar assay methodologies.

Seroprevalence data was evaluated for each study. The observed seroprevalence using the STRATIFY JCV® DxSelect™ assay was 59% (467/792) in the STRATIFY-2 study and 55% (296/538) in the AFFIRM Study. These seroprevalence values are consistent with the seroprevalence values observed during the clinical studies, when the relationship between JCV serological status and PML development were described. Additionally the STRATIFY JCV® DxSelect™ assay demonstrated 100% concordance with 31 pre-PML samples.

Table 4: Demographic Data and JCV Antibody Prevalence for MS Patients

	AFFIRM (N=538)	STRATIFY-2 (N=792)
Age (years)		
• Range	18-50	19-78
• Mean	35.8	46.4
• Median	36	46
Gender (%)*		
• Male	173/538 (32.2%)	202/792 (25.5%)
• Female	365/538 (67.8%)	590/792 (74.5%)
Geography	North America and EU/Rest of World	US
JCV Antibody Positivity Rate[1] (95% CI)		
• % JCV Antibody Positive	297/538 (55.2%) (51.0 to 59.4)	467/792 (59.0%) (55.5 to 62.3)
• % JCV Antibody Negative	241/538 (44.8%) (40.6 to 49.0)	325/792 (41.0%) (37.7 to 44.5)

1. A total of 10.4% of the samples tested in the AFFIRM study and 16.4% of the samples tested in the STRATIFY-2 study were indeterminate in the DETECTION ASSAY.

Reproducibility

The reproducibility of the assay was assessed using a protocol based on a CLSI guideline for estimating precision of an assay. The protocol consisted of testing three replicates of each panel member for two runs a day for a total of five days at two external testing sites an one internal site. The reproducibility testing panel consisted of four levels of sera and four levels of plasma (EDTA) samples and the low and high positive controls. The four levels included a negative, low, indeterminate and moderate positive sample prepared in a serum matrix and a negative, low, indeterminate and moderate positive sample prepared in a plasma matrix. The indeterminate samples were tested simultaneously in the Confirmation Assay and the Detection Assay.

Table 5: Reproducibility

Parameter	Sample Matrix	Sample Name	Qualitative Results					Quantitative Results											
										Variability Components									
										Between Sites		Between Days		Between Runs/Operators		Within Assay (Repeatability)		Total	
			ND	I	D	NV	Total	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
OD		Cut-Off						120	1.131	0.145	12.8	0.067	5.9	0.088	7.8	0.037	3.2	0.186	16.5
INDEX	Controls	Negative Control	89			1	90	89	0.102	0.010	9.5	0.007	7.1	0.010	9.7	0.006	6.1	0.017	16.5
		Positive Control			90		90	90	1.202	0.008	0.7	0.010	0.8	0.022	1.8	0.036	3.0	0.044	3.7
	Plasma	Indeterminate		90			90	90	0.270	0.010	3.7	0.009	3.4	0.011	4.2	0.014	5.1	0.023	8.3
		Low positive		39	51		90	90	0.410	0.000	0.0	0.000	0.0	0.022	5.5	0.023	5.7	0.032	7.9
		Medium positive			90		90	90	0.858	0.041	4.7	0.025	3.0	0.018	2.1	0.059	6.9	0.078	9.1
		Negative	89			1	90	89	0.129	0.002	1.8	0.007	5.5	0.013	9.8	0.008	5.8	0.017	12.8
	Serum	Indeterminate		90			90	90	0.283	0.012	4.3	0.000	0.0	0.011	3.9	0.018	6.3	0.024	8.5
		Low positive		18	72		90	90	0.422	0.004	0.9	0.004	1.1	0.018	4.2	0.013	3.1	0.023	5.4
		Medium positive			90		90	90	1.055	0.048	4.5	0.000	0.0	0.041	3.9	0.045	4.3	0.078	7.4
		Negative	84	2		4	90	86	0.109	0.024	22.3	0.000	0.0	0.018	16.5	0.013	11.7	0.033	30.1
%Inhibition	Plasma	Indeterminate	11		77	2	90	88	51.05	2.006	3.9	0.000	0.0	3.648	7.1	3.357	6.6	5.348	10.5
	Serum	Indeterminate	2		85	3	90	87	62.59	3.889	6.2	2.438	3.9	0.000	0.0	5.550	8.9	7.202	11.5

ND = Not Detected, I = Indeterminate, D = Detected, NV = Invalid

Reproducibility at the Lower Cut Point

In order to demonstrate precision near the lower cut point, two contrived samples, one sera and one plasma (EDTA) were prepared to be near the lower cut-point of the assay. Each sample was diluted twenty times and tested at one internal site in the Detection Assay and the Confirmation Assay. As depicted in the following table the %CV was ≤ 2.6%.

Table 6: Reproducibility at the Lower Cut Point

Sample Matrix	Descriptive Statistics of Detection Assay (Index)						Descriptive Statistics of Confirmation Assay (%Inhibition)					
	N	Min	Max	Mean	SD	%CV	N	Min	Max	Mean	SD	%CV
Plasma	20	0.33	0.36	0.34	0.01	2.3	20	61.66	66.97	65.11	1.43	2.2
Serum	19*	0.19	0.21	0.2	0.01	2.6	19	55.89	60.92	58	1.16	2

*One replicate was Invalid

Cross Reactivity

Cross reactivity was evaluated in a three part study conducted at an internal testing site. Part one of the study evaluated cross reactivity with commercially available human antibodies spiked into JCV negative serum and plasma as determined by the STRATIFY JCV® DxSelect™ assay. The potentially cross reacting antibodies were spiked in at concentrations estimated to be approximately 2-4 times higher than the estimated limit of detection of JCV antibody. Each antibody tested was not detected with the STRATIFY JCV® DxSelect™ assay. There was no observed reactivity with the three potentially cross-reacting antibodies tested in part one of the study.

Table 7: Cross Reactivity - Part One – Spiked Antibodies

Cross Reactant	Concentration	Number Detected	
		Serum	Plasma
Antibody to *Escherichia coli*	0.4 µg/mL	0/3	0/3
Antibody to *Mycobacterium tuberculosis*	0.4 µg/mL	0/3	0/3
Antibody to *Pneumocystis jiroveci*	unquantified	0/3	0/3

In part two of the study the study panel consisted of a least twenty remnant specimens that previously tested positive for each potential cross reacting antibody. Each member of the panel was evaluated using the STRATIFY JCV® DxSelect™ assay along with appropriate controls. The seroprevalence of JCV for each group of potential cross reactants in the panel was compared to the expected seroprevalence of JCV in the normal population. If a group demonstrated a higher than expected seroprevalence of JCV (>70%) it may be an indicator of potential cross reactivity. Four groups of patients (*C. pneumoniae,* HIV, CMV, HSV 1*)* exhibited a positivity rate that was slightly above that observed in previously reported studies. The results suggest that these groups demonstrate potential cross reactivity.

Table 8: Cross Reactivity – Part Two – Sero-prevalence Comparison

Sample Matrix	Cross Reactant	Total No. of Replicates or Samples	Screening Result Count (Based on Index)			Confirmation Result Count (Based on %Inhibition)			Final Interpretation			
									Count		%	
			D	IND	ND	Not Tested	D	ND	D	ND	D	ND
Unknown	HIV	22	16	2	4	20	1	1	17	5	77.3	22.7
Serum	*C. pneumoniae*	48	35	5	8	43	2	3	37	11	77.1	22.9
	C. trachomatis	20	11	2	7	18	1	1	12	8	60.0	40.0
	CMV	40	27	7	6	33	5	2	32	8	80.0	20.0
	Candida	40	26	6	8	34	1	5	27	13	67.5	32.5
	EBV	40	23	9	8	31	2	7	25	15	62.5	37.5
	HSV 1	48	32	6	10	42	2	4	34	14	70.8	29.2
	HSV 2	20	8	5	7	15	1	4	9	11	45.0	55.0
	HHV 6	20	11	2	7	18		2	11	9	55.0	45.0
	Listeria	17	8	3	6	14		3	8	9	47.1	52.9
	Mycoplasma	20	10	4	6	16		4	10	10	50.0	50.0
	Treponema pallidum	19	12	2	5	17	1	1	13	6	68.4	31.6
	VZV	20	12	4	4	16	1	3	13	7	65.0	35.0
All		**334**	**208**	**48**	**78**	**286**	**15**	**33**	**223**	**111**	**66.8**	**33.2**

D= Detected, ND = Not Detected, IND = Indeterminate

Part three of the cross reactivity evaluation consisted of an evaluation of the potential cross reactivity with other polyoma viruses. This included a cross absorption study using BKV virus like particles (VLP). A total of 40 clinical specimens that have previously tested positive for JCV antibodies in the STRATIFY JCV® DxSelect™ assay were tested in a confirmation style assay using JCV VLP and BKV VLP. A control set consisted of samples spiked with JCV VLP at the same concentration of VLP that is used in the confirmation assay. The test samples were with spiked with BKV VLP at the same concentration. The tests samples would be considered to be cross reactive if the % change in signal between the unspiked sample and the sample spiked with BKV VLP is > 45%. The control set demonstrated % change in OD that was consistent with expectations. The test samples demonstrated % change in OD values due to BKV VLP that ranged from -15 to 27%. No samples exhibited >45% change in the OD signal when spiked with BKV VLP, indicating that the assay does not cross react with BKV.

Additional analysis of the structure of the VP1 protein of other polyoma viruses indicates that BKV and JCV are more closely related (79.4% similarity) than other polyoma virus such as Merkel Cell virus and WU virus and KI virus (30.8 to 50.8 % similarity). Due to the differences in viral structure a similar experiment with other polyoma virus VLP was not conducted.

Interferences

Potential interference due to endogenous substances were evaluated using a protocol based on a CLSI guideline for estimating the effect of interfering substances. The testing panel consisted of sera and plasma samples that contain JCV antibody with an index value that is close to the assay cut off. The samples are spiked with the potential interferent at the highest possible endogenous level and compared to baseline testing of the same serum and plasma samples that did not contain the interferent. For all of the potential interferents with the exception of γ globulin; the observed differences in signal did not cause any changes in interpretation of the final result. A potentially interference is suspected if the % Change from the baseline sample is > 20%. Commercially available γ globulin is produced using normal human serum containing IgG antibodies, since the seroprevalence of antibodies to JCV virus is approximately 55% in the normal population it is expected to react with this assay.

Table 9: Interference Summary – Signal Comparison to Baseline

Substance Name	Substance Concentration	Plasma			Serum		
		Average Index		%Change from Baseline	Average Index		%Change from Baseline
		Baseline Sample	Interference Sample		Baseline Sample	Interference Sample	
Albumin	120 mg/mL	0.35	0.38	8.6	0.43	0.37	-14.0
Ascorbic Acid	0.03 mg/mL	0.40	0.42	5.0	0.42	0.44	4.8
Bilirubin	0.2 mg/mL	0.33	0.33	0.0	0.42	0.38	-9.5
Cholesterol	5 mg/mL	0.48	0.40	-16.7	0.46	0.44	-4.3
Gamma Globulin	60 mg/mL	0.38	3.54	831.6	0.41	3.52	758.5
Hemoglobin	110 mg/mL	0.36	0.41	13.5	0.44	0.46	5.0
Hemoglobin	165 mg/mL		0.38	6.1		0.39	-10.1
Hemoglobin	220 mg/mL		0.35	-2.4		0.37	-16.0
Triglycerides	10 mg/mL	0.36	0.35	-2.8	0.40	0.37	-7.5

Note: Three dilution of Gamma Globulin stock solution exhibited Index values - 3.22, 3.57 and 3.40.

HOOK EFFECT

The Hook Effect was investigated during the optimization of the JCV VLP coating concentration and conjugate dilution using a panel of serum samples ranging from negative reactivity to strong positive reactivity (beyond the limit of the plate reader at OD_{450} – 4.000) to JCV. No Hook Effect was observed at the optimal coating VLP concentration and conjugate dilution for this assay using the panel tested.

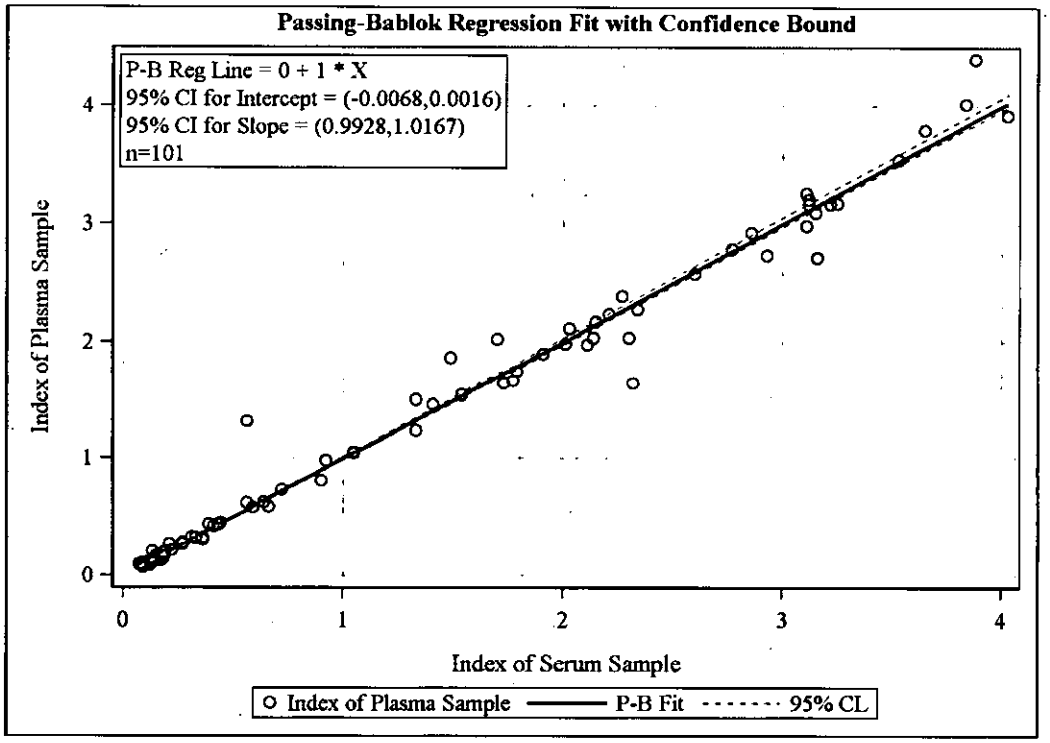
SAMPLE MATRIX COMPARISON

A panel of 109 paired sera and plasma samples (EDTA) were evaluated in the STRATIFY JCV® DxSelect™ assay. Of the total 109 pairs one pair was eliminated from the regression analysis because the result of the sera sample was invalid, of the remaining 108 pairs 7 pairs were qualitatively detected with OD values that exceeded the range of the spectrophotometer. These pairs of samples are included in the qualitative result comparison below, but eliminated from the regression analysis since an index value could not be calculated. Passing-Bablok regression analysis of the pairs of sera and plasma specimens demonstrates a slope of 1 with a 95% CI of (0.9928 to 1.0167)) and an intercept of 0 with a 95% CI of (-0.0068 to 0.0016). Analysis of the qualitative results yielded Positive Percent Agreement = 96.7% (58/60), 95% CI: (88.6 to 99.1%), and Negative Percent Agreement = 97.9% (47/48), 95%CI: (89.1 to 99.6%)

Table 10: Sample Matrix Comparison - Sera vs. Plasma Qualitative Results

Final Plasma Result	Final Serum Result (Count)			All
	ND	D	Invalid	
ND	47	2	1	50
D	1	58		59
All	**48**	**60**	**1**	**109**

Figure 1: Passing-Bablok Regression Analysis (Sera vs. Plasma)



Sample Comparison -Fresh vs. Frozen

A panel of 53 pairs of fresh and frozen plasma specimens (EDTA) and 53 pairs of fresh and frozen sera specimens were evaluated in the STRATIFY JVC DxSelect assay. Of the total 106 pairs, two serum pairs and three plasma pairs were excluded from the regression analysis due to having OD readings above the spectrophotometers measuring range. One pair of serum samples was excluded from the regression analysis due to an invalid result for the fresh specimen. Passing-Bablok regression analysis of the pairs of fresh vs. frozen specimens demonstrates a slope of 1 with a 95% CI of (0.9900 to 1.0221) and an intercept of 0 with a 95% CI of (-0.0090 to 0.0028). Analysis of the qualitative results yielded a Positive

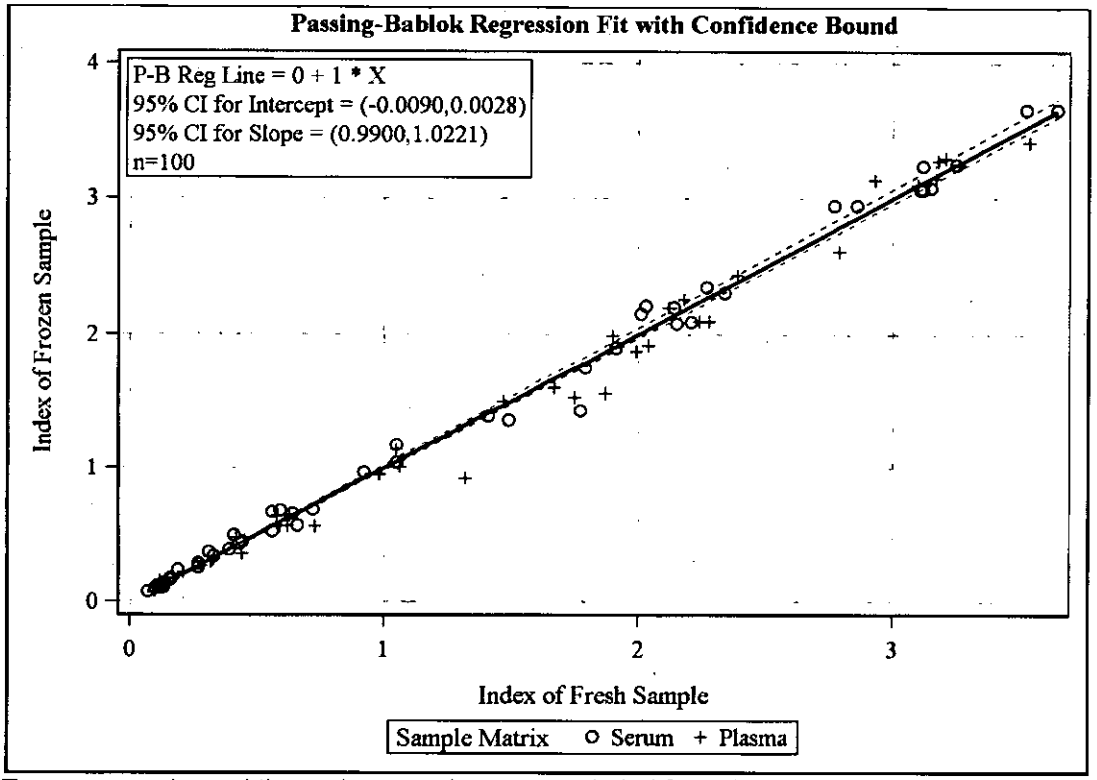
Percent Agreement = 96% (72/75), 95% CI: (88.9 to 98.6%) and Negative Percent Agreement = 96.7% (29/30), 95%CI: (83.3 to 99.4%).

Table 11: Sample Comparison -Fresh vs. Frozen

		Final Frozen Result (Count)		
Sample Matrix	**Final Fresh Result**	**ND**	**D**	**All**
Plasma	**ND**	16		16
	D	1	36	37
	All	17	36	53
Serum	**ND**	13	1	14
	D	2	36	38
	NV	1		1
	All	16		16
All		32	68	106

ND = Not Detected, D = Detected; NV = Invalid
* Three plasma pairs and two serum pairs had results that were above the range of the spectrophotometer, these samples are considered detected.

Figure 2: Sample Comparison –Regression Analysis Fresh vs. Frozen



Note: Two serum pairs and three plasma pairs were excluded from the regression analysis due to having OD readings above the spectrophotometers measuring range. One pair of serum samples was excluded from the regression analysis due to an invalid result for the fresh specimen.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

AUG 16 2012

Focus Diagnostics, Inc.
C/O Tara Viviani
11331 Valley View Street
Cypress, California 90630

Re: K120986
Trade/Device Name: STRATIFY JCV® DxSelect™
Regulation Number: 21 CFR 866.3336
Regulation Name: John Cunningham Virus serological reagents
Regulatory Class: Class II Special Controls
Product Code: OYP
Dated: August 3, 2012
Received: August 6, 2012

Dear Ms. Viviani:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

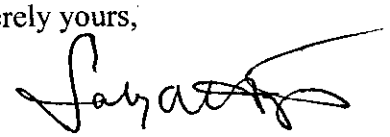
Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice

requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,



Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): k120986

Device Name: STRATIFY JCV® DxSelect™

Indications for Use:

The Focus Diagnostics' STRATIFY JCV® DxSelect™ assay is intended for the qualitative detection of antibodies to JC virus in human serum or plasma. The assay is intended for use in conjunction with other clinical data, in multiple sclerosis patients receiving or considering natalizumab therapy, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only.

The assay is not intended for donor screening. The performance of this assay has not been established for use in other immunocompromised patient populations or patients with different disease conditions or undergoing other treatments or in neonates and pediatric patient populations.

Prescription Use X (Part 21 CFR 801 Subpart D) And/Or Over-The-Counter Use ___ (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Tamara Feldblum

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K120986